## AMENDMENT

**In the Specification:**

Please amend the title to read as follows:

--Polynucleotides Encoding the *fkbA* Gene of the FK-520 Polyketide Synthase Gene Cluster--

Please replace the paragraph beginning at page 26, line 17 with the following rewritten paragraph:

--Referring to Figures 1 and 3, the FK-520 PKS gene cluster is composed of four open reading frames designated *fkbB*, *fkbC*, *fkbA*, and *fkbP*. The *fkbB* open reading frame encodes the loading module and the first four extender modules of the PKS. The *fkbC* open reading frame encodes extender modules five and six of the PKS. The *fkbA* open reading frame encodes extender modules seven, eight, nine, and ten of the PKS. The *fkbP* open reading frame encodes the NRPS of the PKS. Each of these genes can be isolated from the cosmids of the invention described above. The DNA sequences of these genes are provided below (SEQ ID NO:1) preceded by the following table identifying the start and stop codons of the open reading frames of each gene and the modules and domains contained therein.

| Nucleotides | Gene or Domain |
|---|---|
| complement (412 - 1836) | *fkbW* |
| complement (2020 - 3579) | *fkbV* |
| complement (3969 - 4496) | *fkbR2* |
| complement (4595 - 5488) | *fkbR1* |
| 5601 - 6818 | *fkbE* |
| 6808 - 8052 | *fkbF* |
| 8156 - 8824 | *fkbG* |
| complement (9122 - 9883) | *fkbH* |
| complement (9894 - 10994) | *fkbI* |
| complement (10987 - 11247) | *fkbJ* |
| complement (11244 - 12092) | *fkbK* |
| complement (12113 - 13150) | *fkbL* |
| complement (13212 - 23988) | *fkbC* |
| complement (23992 - 46573) | *fkbB* |
| 46754 - 47788 | *fkbO* |
| 47785 - 52272 | *fkbP* |

| | |
|---|---|
| 52275 - 71465 | *fkbA* (SEQ ID NO:72) |
| 71462 - 72628 | *fkbD* |
| 72625 - 73407 | *fkbM* |
| complement (73460 - 76202) | *fkbN* |
| complement (76336 - 77080) | *fkbQ* |
| complement (77076 - 77535) | *fkbS* |
| complement (44974 - 46573) | CoA ligase of loading domain |
| complement (43777 - 44629) | ER of loading domain |
| complement (43144 - 43660) | ACP of loading domain |
| complement (41842 - 43093) | KS of extender module 1 (KS1) |
| complement(40609 - 41842) | AT1 |
| complement (39442 - 40609) | DH1 |
| complement (38677 - 39307) | KR1 |
| complement (38371 - 38581) | ACP1 |
| complement (37145 - 38296) | KS2 |
| complement (35749 - 37144) | AT2 |
| complement (34606 - 35749) | DH2 (inactive) |
| complement (33823 - 34480) | KR2 |
| complement (33505 - 33715) | ACP2 |
| complement (32185 - 33439) | KS3 |
| complement (31018 - 32185) | AT3 |
| complement (29869 - 31018) | DH3 (inactive) |
| complement (29092 - 29740) | KR3 |
| complement (28750 - 28960) | ACP3 |
| complement (27430 - 28684) | KS4 |
| complement (26146 - 27430) | AT4 |
| complement (24997 - 26146) | DH4 (inactive) |
| complement (24163 - 24373) | ACP4 |
| complement (22653 - 23892) | KS5 |
| complement (21420 - 22653) | AT5 |
| complement (20241 - 21420) | DH5 |
| complement (19464 - 20097) | KR5 |
| complement (19116 - 19326) | ACP5 |
| complement (17820 - 19053) | KS6 |
| complement (16587 - 17820) | AT6 |
| complement (15438 - 16587) | DH6 |
| complement (14517 - 15294) | ER6 |
| complement (13761 - 14394) | KR6 |
| complement (13452 - 13662) | ACP6 |
| 52362 - 53576 | KS7 |
| 53577 - 54716 | AT7 |
| 54717 - 55871 | DH7 |
| 56019 - 56819 | ER7 |
| 56943 - 57575 | KR7 |
| 57711 - 57920 | ACP7 |
| 57990 - 59243 | KS8 |

| | |
|---|---|
| 59244 - 60398 | AT8 |
| 60399 - 61412 | DH8 (inactive) |
| 61548 - 62180 | KR8 |
| 62328 - 62537 | ACP8 |
| 62598 - 63854 | KS9 |
| 63855 - 65084 | AT9 |
| 65085 - 66254 | DH9 |
| 66399 - 67175 | ER9 |
| 67299 - 67931 | KR9 |
| 68094 - 68303 | ACP9 |
| 68397 - 69653 | KS10 |
| 69654 - 70985 | AT10 |
| 71064 - 71273 | ACP10 -- |

a1

Please replace the paragraph beginning on page 77, line 3 with the following rewritten paragraph:

--To construct a hybrid PKS or FK-520 derivative PKS gene of the invention, one can employ a technique, described in PCT Pub. No. 98/27203 and U.S. patent application Serial No. 08/989,332, filed 11 Dec. 1997, now U.S. Patent No. 6,033,883, each of which is incorporated herein by reference, in which the large PKS gene is divided into two or more, typically three, segments, and each segment is placed on a separate expression vector. In this manner, each of the segments of the gene can be altered, and various altered segments can be combined in a single host cell to provide a recombinant PKS gene of the invention. This technique makes more efficient the construction of large libraries of recombinant PKS genes, vectors for expressing those genes, and host cells comprising those vectors--

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Please replace the paragraph beginning on page 80, line 8 with the following rewritten paragraph:

--For 2-hydroxymalonyl CoA biosynthesis, the *fkbH*, *fkbI*, *fkbJ*, and *fkbK* genes are sufficient to confer this ability on *Streptomcyces* host cells. For conversion of 2-hydroxymalonyl to 2-methoxymalonyl, the *fkbG* gene is also employed. While the complete coding sequence for *fkbH* is provided on the cosmids of the invention, the sequence for this gene provided herein may be missing a T residue, based on a comparison made with a similar gene cloned from the

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ansamitocin gene cluster by Dr. H. Floss. Where the sequence herein shows one T, there may be two, resulting in an extension of the *fkbH* reading frame to encode the amino acid sequence (SEQ ID NO:2):

Please replace the paragraph beginning on page 86, line 25 with the following rewritten paragraph:

To construct an expression cassette for performing module 8 AT domain replacements in the FK-520 PKS, a 4.6 kb *Sph*I fragment from the FK-520 gene cluster was cloned into plasmid pLitmus 38 (a cloning vector available from New England Biolabs). The 4.6 kb *Sph*I fragment, which encodes the ACP domain of module 7 followed by module 8 through the KR domain, was isolated from an agarose gel after digesting the cosmid pKOS65-C31 with *Sph* I. The clone having the insert oriented so the single *Sac*I site was nearest to the *Spe*I end of the polylinker was identified and designated as plasmid pKOS60-21-67. To generate appropriate cloning sites, two linkers were ligated sequentially as follows. First, a linker was ligated between the *Spe*I and *Sac*I sites to introduce a *Bgl*II site at the 5' end of the cassette, to eliminate interfering polylinker sites, and to reduce the total insert size to 4.5 kb (the limit of the phage KC515). The ligation reactions contained 5 picomolar unphosphorylated linker DNA and 0.1 picomolar vector DNA, i.e., a 50-fold molar excess of linker to vector. The linker had the following sequence (SEQ ID NOS:3-4):

5'-CTAGTGGGCAGATCTGGCAGCT-3'
3'-ACCCGTCTAGACCG-5'

The resulting plasmid was designated pKOS60-27-1.

Please replace the paragraph beginning on page 87, line 12 with the following rewritten paragraph:

Next, a linker of the following sequence was ligated between the unique *Sph*I and *Afl*II sites of plasmid pKOS60-27-1 to introduce an *Nsi*I site at the 3' end of the module 8 cassette. The linker employed was (SEQ ID NOS:5-6):

5'-GGGATGCATGGC-3'
3'-GTACCCCTACGTACCGAATT-5'

The resulting plasmid was designated pKOS60-29-55.--

Please replace the paragraph beginning on page 87, line 18 with the following rewritten paragraph:

--To allow in-frame insertions of alternative AT domains, sites were engineered at the 5' end (*Avr* II or *Nhe* I) and 3' end (*Xho* I) of the AT domain using the polymerase chain reaction (PCR) as follows. Plasmid pKOS60-29-55 was used as a template for the PCR and sequence 5' to the AT domain was amplified with the primers SpeBgl-fwd and either Avr-rev or Nhe-rev: (SEQ ID NOS:7-9)

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SpeBgl-fwd 5'-CGACTCACTAGTGGGCAGATCTGG-3'

Avr-rev 5'-CACGCCTAGGCCGGTCGGTCTCGGGCCAC-3'

Nhe-rev 5'-GCGGCTAGCTGCTCGCCCATCGCGGGATGC-3'--

Please replace the paragraph beginning on page 88, line 6 with the following rewritten paragraph:

--Plasmid pKOS60-29-55 was again used as a template for PCR to amplify sequence 3' to the AT domain using the primers BsrXho-fwd and NsiAfl-rev (SEQ ID NOS:10-11):

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BsrXho-fwd 5'-GATGTACAGCTCGAGTCGGCACGCCCGGCCGCATC-3'

NsiAfl-rev 5'-CGACTCACTTAAGCCATGCATCC-3'--

Please replace the paragraph beginning on page 88, line 16 with the following rewritten paragraph:

--Malonyl and methylmalonyl-specific AT domains were cloned from the rapamycin cluster using PCR amplification with a pair of primers that introduce an *Avr*II or *Nhe*I site at the 5' end and an *Xho*I site at the 3' end. The PCR conditions were as given above and the primer sequences were as follows (SEQ ID NOS:12-15):

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RATN1 5'-ATCCTAGGCGGGCRGGYGTGTCGTCCTTCGG-3'
(3' end of Rap KS sequence and universal for malonyl and methylmalonyl CoA),
RATMN2 5'-ATGCTAGCCGCCGCGTTCCCCGTCTTCGCGCG-3'
(Rap AT shorter version 5'- sequence and specific for malonyl CoA),
RATMMN2 5'-ATGCTAGCGGATTCGTCGGTGGTGTTCGCCGA-3'
(Rap AT shorter version 5'- sequence and specific for methylmalonyl CoA), and
RATC 5'-ATCTCGAGCCAGTASCGCTGGTGYTGGAAGG-3'
(Rap DH 5'- sequence and universal for malonyl and methylmalonyl CoA).--

Please replace the paragraph beginning on page 89, line 16 with the following rewritten paragraph:

a9 --The *Avr*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 12 of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below. The AT of rap module 12 is specific for incorporation of malonyl units (SEQ ID NOS:16-17).--

Please replace the paragraph beginning on page 92, line 49 with the following rewritten paragraph:

a10 --The *Avr*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 13 (specific for methylmalonyl CoA) of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below (SEQ ID NOS:18-19).--

Please replace the paragraph beginning on page 96, line 28 with the following rewritten paragraph:

a11 --The *Nhe*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 12 (specific for malonyl CoA) of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below (SEQ ID NOS:20-21).--

Please replace the paragraph beginning on page 100, line 1 with the following rewritten paragraph:

a12 --The *Nhe*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 13 (specific for methylmalonyl CoA) of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below (SEQ ID NOS:22-23).--

Please replace the paragraph beginning on page 105, line 24 with the following rewritten paragraph:

a13 --The naturally occurring module 8 sequence for the MA6548 strain is shown below, followed by the illustrative hybrid module 8 sequences for the MA6548 strains (SEQ ID NOS:24-25).--

Please replace the paragraph beginning on page 109, line 21 with the following rewritten paragraph:

a14 --The *Avr*II-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 12 of rapamycin is shown below (SEQ ID NOS:26-27).--

Please replace the paragraph beginning on page 112, line 51 with the following rewritten paragraph:

a15 --The *Avr*II-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 13 of rapamycin is shown below (SEQ ID NOS:28-29).--

Please replace the paragraph beginning on page 116, line 32 with the following rewritten paragraph:

a16 --The *Nhe*I-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 12 of rapamycin is shown below (SEQ ID NOS:30-31).--

Please replace the paragraph beginning on page 120, line 11 with the following rewritten paragraph:

a17 --The *Nhe*I-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 13 of rapamycin is shown below (SEQ ID NOS:32-33).--

Please replace the paragraph beginning on page 125, line 1 with the following rewritten paragraph:

a18 --The following table shows the location and sequences surrounding the engineered site of each of the heterologous AT domains employed (SEQ ID NOS:34-63, in order of appearance). The FK-506 hybrid construct was used as a control for the FK-520 recombinant cells produced, and a similar FK-520 hybrid construct was used as a control for the FK-506 recombinant cells.--

Please replace the paragraph beginning on page 126, line 1 with the following rewritten paragraph:

a19 The sequences shown below provide the location of the KS/AT boundaries chosen in the FK-520 module 8 coding sequences. Regions where *Avr*II and *Nhe*I sites were ~~engineered~~ are indicated by lower case and underlining (SEQ ID NOS:64-65).

Please replace the paragraph beginning on page 126, line 21 with the following rewritten paragraph:

a20 The sequences shown below provide the location of the AT/DH boundary chosen in the FK-520 module 8 coding sequences. The region where an *Xho*I site was engineered is indicated by lower case and underlining (SEQ ID NOS:66-67).

Please replace the paragraph beginning on page 126, line 29 with the following rewritten paragraph:

a21 The sequences shown below provide the location of the KS/AT boundaries chosen in the FK-506 module 8 coding sequences. Regions where *Avr*II and *Nhe*I sites were engineered are indicated by lower case and underlining (SEQ ID NOS:68-69).

Please replace the paragraph beginning on page 127, line 4 with the following rewritten paragraph:

a22 The sequences shown below provide the location of the AT/DH boundary chosen in the FK-506 module 8 coding sequences. The region where an *Xho*I site was engineered is indicated by lower case and underlining (SEQ ID NOS:70-71).